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ANALYSIS OF TRACE VX IN ACIDIFIED VX HYDROLYSATE SAMPLES

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14. ABSTRACT

The objective of this study was to modify and optimize US Army Element, Assembled Chemical Weapons Alternatives (USAE ACWA) method BGCAPP-204 (VX in Caustic Hydrolysate by Cool on-Column GC/MS) for analysis of ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX) in acidified VX hydrolysate matrices. The modified procedures included the injection of VX spikes directly to the acidified hydrolysate samples and the use of an alkaline buffer (pH approximately 12) to adjust the samples to a pH close to 11 prior to VX extraction. The applicability of the modified method was tested and successfully demonstrated for VX hydrolysate alone and in the presence of energetics hydrolysate over a sample pH range of 5 to 9. VX extraction recoveries were ~80%. No VX reformation was observed over the pH range studied.

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PREFACE

The work described in this report was authorized by the U.S. Army Element, Assembled Chemical Weapons Alternatives (USAE ACWA) under Job Order Numbers 8VEDDR and 8VEZDR. The work was started in November 2007 and completed in November 2008.

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ANALYSIS OF TRACE VX IN ACIDIFIED VX HYDROLYSATE SAMPLES

1. INTRODUCTION

A mission of the US Army Element, Assembled Chemical Weapons Alternatives (USAE ACWA) program is to develop alternative methods to incineration to safely destroy the chemical weapon stockpiles stored at 2 sites: Pueblo, Colorado and Blue Grass, Kentucky. At Blue Grass, where a pilot plant called the Blue Grass Chemical Agent Destruction Pilot Plant (BGCAPP) is being designed and constructed, the method for ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX) destruction is caustic neutralization with NaOH in a well-mixed reactor at 90 °C.[1] To satisfy the required VX destruction level of >99.9999% in the reactor, a reliable analytical method is necessary to detect low levels of VX in the reactor final product mixture (also called VX hydrolysate) to provide clearance at an action level of 160 ng/mL [parts per billion (ppb)]. A robust gas chromatograph – mass selective detector (GC-MSD) analytical method (BGCAPP-204) was developed for this purpose and fully tested at Battelle-Columbus.[2] The method involves the addition of an acidic buffer, KH_2PO_4/NaCl (pH 4), to the hydrolysate to reduce the sample pH to about 12, extraction with hexane, C2 solid phase extraction (SPE) cleanup of the water-washed hexane extract using 90:10 CH_2Cl_2:isopropanol (IPA) as the elution solvent, and analysis by GC/MSD in the selective ion mode using cool on-column injection. Quantitation is accomplished using naphthalene-d8 as an internal standard. The VX detection limit using this method is typically <20 ng/mL.[1,2]

At BGCAPP, the overall design involves destruction of both chemical agent and energetics. The process includes initial separation of agent and energetics from the munitions, caustic hydrolysis of each, and blending the VX hydrolysate with aluminum-precipitated energetics hydrolysate (1:2.5 w:w ratio) for subsequent treatment in the SCWO (supercritical water oxidation) reactors. Prior to feeding the blended hydrolysate mixture from the SCWO blend tank to the SCWO reactors, chloride and sulfate anions are added to the blend tank in the appropriate ratio (Cl:S ratio of 1.08:1), such that the corresponding sodium salts can be transported as fluid in the reactor under the SCWO processing conditions. Current design calls for adding these elements as 35% HCl, 93% H_2SO_4 and sodium chloride. The procedures and time intervals for additions to the SCWO blend tank are in the following order: 25,715 lbs energetics hydrolysate, 10,285 lbs VX hydrolysate, 464 lbs 35% HCl (~40 min), 242 lbs 93% H_2SO_4 (~40 min) and 342 lbs solid sodium chloride (~40 min).

Because the target pH of the energetics hydrolysate after aluminum filtration is 8 and HCl and H_2SO_4 are further added to the blend, the pH of the mixture is lowered during the mixing steps. This leads to two questions: (1) can the method for clearance of VX in caustic hydrolysate be adapted for detection in lower pH matrices and in the presence of energetics hydrolysate and (2) does VX reformation occur in acidified VX and/or VX/energetics hydrolysate? To address the first question and apply the existing method, the pH of the acidified VX hydrolysate sample must be adjusted to

alkaline in order to extract VX into n-hexane. Initial scoping studies performed at Battelle used 10% NaOH to raise the acidified VX hydrolysate pH to 12 to 13. Inconsistent and low VX spike recoveries were obtained which were attributed to inefficient control of the solution pH, leading to caustic hot spots that can accelerate VX spike degradation.

The primary objective of the investigative study performed here was to modify the existing method and test its suitability for detection of trace levels of VX in acidified VX hydrolysate alone and in the presence of energetics hydrolysate. It has been reported that the major VX degradation products in the VX hydrolysate, MeP(O)(OEt)OH (ethyl methylphosphonate or EMPA), and $HSCH_2CH_2N(iPr)_2$ (VX thiol) may react in the presence of dicyclohexylcarbodiimide (DCC) via a pyrophosphonate intermediate to reform VX.[3] Since pyrophosphonate formation is reported to be most favorable under acidic conditions, VX reformation in acidified VX hydrolysate may be a concern. Therefore, a secondary objective of this study was to use this modified method to investigate the presence of VX reformation in these acidified samples.

Particular attention was paid in this study to extraction pH, which plays a critical role in effective detection of VX. Since VX is only extractable in the neutral, unprotonated form, the pH must be high enough to ensure VX is not protonated. The pK_a of VX first reported in literature was 8.6 at 25 °C.[4,5] More recently, a new value was reported by Van der Schans and coworkers to be 9.4 at 25 °C.[6]

For good spike recovery, the extraction pH must be 1 to 2 units above the pK_a value but must be kept below a value where caustic degradation occurs within the extraction timeframe. Previously reported values of half-lives for VX-NaOH reaction are 40 days at pH 7, 17 hr at pH 11, 2 hr at pH 12, 12 min at pH 13, and 1.3 min at pH 14 (25 °C).[5,7] These values were obtained by indirect methods using extraction followed by GC-MSD or using pH titrations, which were difficult because VX can be protonated at lower pH ranges. In the 1990s, solutions at low VX concentrations in the presence of excess NaOH were directly monitored by ^{31}P or ^{1}H NMR and the rates at constant pH were determined.[8] These observed half-lives, shown in Table 1, indicate that VX reacts with NaOH slowly at pH 11 or below at room temperatures.

Table 1. Observed VX Hydrolysis Rate Data[8]

Temp, °C	$[VX]_o$, M	$[NaOH]_o$, M	pH	k_{obs}, s^{-1}	$t_{½}$	NMR Monitoring
23.0	1×10^{-4}	1×10^{-3}	11	9.23×10^{-6}	21 hr	^{1}H
25.3	1×10^{-3}	1×10^{-2}	12	5.79×10^{-5}	3.3 hr	^{1}H
22.0	1×10^{-2}	0.10	13	3.73×10^{-4}	31 min	^{31}P
22.0	1×10^{-2}	1.25	~14	-	<2 min[a]	^{31}P

[a] A slight solubility problem occurred and reaction was too fast for accurate rate determination.

Three alkaline buffers at pH close to 12 were evaluated during this study in an effort to provide effective pH control of the samples and to improve VX spike recovery. In addition, because the hydrolysate is acidified, the VX spike was added directly to the hydrolysate rather than to the buffer to provide better spike solubility and stability. The modified method was tested in both acidified VX hydrolysate alone and acidified VX/energetics hydrolysate blend over a hydrolysate pH range of 5-9 to investigate method applicability and VX reformation.

2. EXPERIMENTAL PROCEDURES

2.1 Materials

Tribasic potassium phosphate (K_3PO_4, >98%, lot 097K0010), sodium carbonate (Na_2CO_3, lot 18179AB), sodium chloride (NaCl, >99.5%, lot 087K0181) and 2-diethylaminoethanethiol (DEAET) hydrochloride (96%, lot 05114AD) were purchased from Sigma-Aldrich (St. Louis, MO). Anhydrous sodium sulfate (Na_2SO_4, >99%, lot B51580) was purchased from J.T. Baker (Phillipsburg, NJ). Dichloromethane (GC^2 grade, lot CM042), water (HPLC grade, lot CR704), IPA (HPLC grade, lot CQ161) and acetone (GC^2 grade, lot BZ055) were purchased from Burdick and Jackson (Muskegon, MI). Hexane (HR-GC grade, lot 46019) was purchased from EMD Chemicals, Inc (Gibbstown, NJ). Concentrated hydrochloric acid (36.5-38%, reagent A.C.S., lot FL030589) was purchased from Fisher Scientific (Fair Lawn, NJ). Concentrated sulfuric acid (95-98%, lot 2876) was purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). Sodium hydroxide (50%, lot SX0597-1) was purchased from E.M. Science (Gibbstown, NJ). Naphthalene-d8 (99 atom % D, lot TV1320) was purchased from Isotec (Miamisburg, OH). VX used for standards was from Newport ton container origin; agent lot NY-1767-A49 (VX10040). GC/MS purity was 91.3%. C2 SPE cartridges (500 mg/4 mL, lot 051200065) were purchased from Alltech Associates (Deerfield, IL).

VX hydrolysate (517523-76-32, run VXDCCWOA-01) used for these studies was prepared by Battelle-Columbus from DCCDI-stabilized VX, was analyzed by the existing method to verify that VX was below 10 ppb prior to shipment, was received 7 Nov 07 at ECBC, and was stored at 4 °C except when in use. The pH of the solution was 13-14 as measured with pH paper.

Energetics hydrolysate filtrate after removal of aluminum (FAC SR01-082-02) used in these studies was prepared by Battelle-Columbus and received 15 May 2008. The energetics materials used for the hydrolysate preparation were never exposed to VX. The hydrolysate was adjusted to pH 8 at Battelle prior to being shipped to Oberlin for Al removal via filtration and contained 528.1 g HCl, 275.6 g H_2SO_4 and 127.8 g NaCl per 1000 mL (1380 g) of energetics hydrolysate prior to filtration. The hydrolysate filtrate was stored at 4 °C except during use.

2.2 Analytical Method

2.2.1 Preparation of Extraction/Buffer Solutions

NaCl solution was prepared by mixing 30 g of NaCl with 200 mL HPLC water and shaking for 1 min. The solution was stored at room temperature.

The SPE extraction solvent was prepared by dilution of 10 mL IPA to 100 mL with methylene chloride. Solvent mixtures were prepared fresh for each series and were stored at room temperature.

Three extraction buffer solutions were evaluated during the course of the VX hydrolysate studies. Each was prepared in HPLC-grade water. The concentrations of the most concentrated phosphate and the carbonate buffers were chosen to approximate the molar concentration of the KH_2PO_4 solution used in the caustic hydrolysate method (BGCAPP-204). Concentrations and pH values measured for each buffer are listed in Table 2. The concentration of buffer used for all subsequent blended studies was 25 g K_3PO_4/100 mL water (pH ~12.5).

Table 2. Buffer Composition and pH

Buffer Concentration (per 100 mL water)	Buffer pH	pH 7 VX Hydrolysate Extraction pH
46.8 g K_3PO_4	12.6	10.8
23.4 g K_3PO_4	12.3	10.5
20 g Na_2CO_3	11.4	9.6

2.2.2 Preparation of Spiking Solutions

A VX stock solution was prepared by diluting 3.5 mg neat VX (91%) to a final volume of 25 mL with hexane in a volumetric flask (127 µg/mL). The spiking solution was prepared by the dilution of 787 µL of the stock solution to 10 mL with IPA (10 µg/mL). Each spiked 2 mL acidified hydrolysate sample was prepared by the addition of 32 µL of the spiking solution to give a hydrolysate VX concentration of 160 ng/mL (1Z). Spiking for the initial carbonate buffer study was done into the buffer. Spiking for all subsequent studies was done directly into the acidified hydrolysate prior to buffer addition.

A stock solution of naphthalene-d8 internal standard (IS) solution was prepared by weighing 10.1 mg (99% atom % D) into a 10 mL volumetric flask and diluting to the mark with acetone (1 mg/mL). The spiking solution was prepared by

placing 42 µL of stock solution into a 10 mL volumetric flask and diluting with acetone (4.2 µg/mL). Internal standard solutions were stored at 4 °C except when in use.

2-Diethylaminoethanethiol was used as a solvent modifier to improve sensitivity and reproducibility.[9] A 22 mL vial was filled with 0.75 g of the hydrochloride salt and 10 mL 0.1M NaOH was added. This mixture was placed in a 125 mL separatory funnel and 10 mL methylene chloride added. After shaking for 30 s, the methylene chloride layer was collected in a 25 mL volumetric flask. This aqueous solution was extracted a second time and the extracts combined. Additional methylene chloride was added to the combined organic fractions to reach a total volume of 25 mL. The solution was dried with sodium sulfate and stored at freezer temperatures. Fresh solutions were prepared for each extraction series.

2.2.3 Preparation of VX Calibration Standards

To provide matrix matching, calibration standards were prepared in acidified VX hydrolysate extracts. Matrix-matched calibration solutions for all studies were prepared beginning with acidified hydrolysate at pH = 7 and by following the steps in Section 2.2.5. Blended analyses were performed using extraction matrices from pH 7 blended hydrolysate. Carbonate buffer (20 g/100 mL water) was used for the initial carbonate studies (section 3.1.1). K_3PO_4 buffer (46.8 g/100 mL water) was used for the method optimization studies (section 3.1.2). K_3PO_4 buffer (25 g/100 mL water) was used for the VX hydrolysate VX reformation study (section 3.1.3) and all energetics hydrolysate and blend studies (section 3.2). The calibration solutions used are shown in Table 3. Each solution was also spiked with 36 µL DEAET solution (solvent modifier) and 25 µL naphthalene-d8 solution (internal standard) to match the sample matrix.

Table 3. VX Calibration Solutions

VX Concentration (ng/mL, ppb)	CH_2Cl_2/IPA Extract Volume (µL)	10 µL/mL VX Spike Volume (µL)
0	1000	0
160	984	16
240	976	24
320	968	32
400	960	40
480	952	48

2.2.4 Hydrolysate Acidification Procedure

VX hydrolysate samples were acidified to the target pH (5, 7, or 9) with drop-wise addition of concentrated H_2SO_4. VX/energetics hydrolysate blended samples were acidified to the target pH (5, 7, 9, or 11) with drop-wise addition of 93% H_2SO_4. Blended samples prepared according to the plant recipe were acidified by sequential addition of 35% HCl, 93% H_2SO_4, and NaCl. PH measurements were obtained using an Oakton pHTstr3 pH meter purchased from Fisher Scientific (Pittsburgh, PA), with calibration using pH 4.01, pH 7.00, and pH 10.00 buffer solutions.

2.2.5 Extraction Procedure

Acidified VX hydrolysate mixtures were extracted according to BGCAPP-204, with two exceptions: (1) K_3PO_4 (pH ~12.5) and Na_2CO_3 (pH 11.4) were used as extraction buffers instead of KH_2PO_4 (pH 4) and (2) after the initial Na_2CO_3 study (section 3.1.1), VX was spiked directly into the acidified hydrolysate rather than into the buffer. The steps performed were as follows:

SPE Cartridge Conditioning

a. A 50 mL empty reservoir was attached to the top of a 500 mg C2 SPE cartridge.
b. Cartridge was conditioned with 15 mL clean hexane.

Extraction of Acidified VX Hydrolysate Mixtures

c. 1 mL of hexane was added to a 125 mL separatory funnel to ensure no leakage.
d. A 2 mL aliquot of homogenized acidified hydrolysate was placed into a 50 mL glass conical tube.
e. If spiked, 32 µL of a 10 µg/mL VX solution was added (160 ng/mL spike) to the hydrolysate.
f. 1.3 mL of buffer solution was added.
g. 6.0 mL of NaCl solution (30 g NaCl/200 mL water) was added and the mixture shaken for 5 to 10 s.
h. The mixture was extracted 3 times by shaking the hydrolysate with 13 mL hexane for 30 s. Each hydrolysate/hexane mixture was poured into the separatory funnel in step c and the aqueous layer drained for repeated extraction with hexane. Total hexane volume was ~40 mL.
i. The hexane extract was washed twice, each time with 10 mL HPLC water, shaking 30 s. The bottom water layers were drained into a waste container.

SPE Cleanup

j. The hexane extract from step i was poured from the separatory funnel into the SPE reservoir and allowed to flow through the SPE column using gravity.
k. Once the upper reservoir was empty it was rinsed with 3 mL hexane.
l. A slight vacuum was applied to pull the final hexane through the cartridge until dripping stopped (not to dryness).
m. The upper reservoir was removed. Any water droplets in the cartridge were removed with a disposable pipet.
n. VX was eluted by adding 2 mL of 90:10 CH_2Cl_2/IPA to the SPE column.
o. Solvent extract was collected using gravity until dripping stopped. A vacuum was used to collect final drops of solvent.
p. CH_2Cl_2/IPA solvent mixture was evaporated to 1 mL using a gentle stream of nitrogen.
q. 36 µL of 22.6 mg/mL DEAET modifier solution and 25 µL 1 mg/mL naphthalene-d8 IS were added to each 1 mL sample.

2.2.6 Instrumentation

Analyses were performed using an Agilent (Wilmington, DE) 5973i MSD equipped with a 6890N GC. Parameters used for analysis of VX extracts were as specified in method BGCAPP-204 and are listed in Tables 4 and 5.

Table 4. GC Parameters

Column	Rtx-1701, 30 m x 0.25 mm, 1.0 µm film (Restek)
Guard column	IP Deactivated 2 m x 0.32 mm (Restek)
Column temperature	50-200 °C (5 min) @ 20 °C /min; 200-230 °C @ 3 °C /min; 230-260 °C @ 30 °C /min; post-run 260 °C (7 min)
Carrier gas	Helium
Flow rate	1.1 mL/min constant flow
Injection mode	Cool on-column
Injection temperature	Tracks oven + 3 °C
Injection volume	1 µL, autoinjection

Table 5. MS Parameters

Acquisition mode	SIM
Ionization mode	EI
Transfer line temperature	280 °C
Source temperature	230 °C
Quads temperature	150 °C
Solvent delay	4 min
Electron energy	70 eV
Multiplier voltage	Autotune + 200 V
Naphthalene-d8 scan ions (0-9 min)	68, 134, 136 (quant ion)
VX scan ions (>9 min)	139 (quant ion), 167, 224, 252
Ion dwell time	100 ms
Scans/s	1.77

GC column conditioning prior to sample analysis improved analytical reproducibility. Some initial drifting of the VX/IS ratio and the VX retention time was observed with a new column. Both parameters stabilized after 9 injections of VX hydrolysate extract. After the first day, in general two extract runs were done daily prior to analysis of a set of samples and was adequate to condition the column and obtain stable, reproducible results.

2.2.7 Quantitation

Quantitation was performed using a six-point IS calibration curve (VX/IS ratio versus VX concentration) with forced origin as specified in method BGCAAP-204. Calibration curves were generated daily for each experimental set. Correlation coefficients (r^2) varied from 0.992 to 0.999. Excellent instrument stability was observed over the course of these studies as verified by check standards run at the end of each series and the day-to-day reproducibility of the calibration curves.

3. RESULTS AND DISCUSSION

3.1 BGCAPP-204 Method Modification in Acidified VX Hydrolysate

3.1.1 Initial Modification Using Carbonate Buffer

Initial studies were performed to investigate (1) VX spike recovery and (2) VX reformation in acidified VX hydrolysate at pH 5, pH 7 and pH 9. 20 g anhydrous sodium carbonate per 100 mL water (pH 11.4) was used as the buffer to raise the pH to an acceptable level for extraction. The VX spike for this study was added directly to the

buffer as specified in method BGCAPP-204. A summary of extraction recoveries obtained is given in Table 6. Consistent recoveries around 70% were obtained. No VX was detected in any of the unspiked acidified hydrolysate samples over the pH 5-9 range.

3.1.2 Method Optimization Studies to Improve Recoveries

The primary objective of this series of experiments was to improve VX recovery. Two approaches were taken. First, phosphate (K_3PO_4, pH ~12.5) buffers were substituted for the carbonate buffer (pH 11.4). The rationale for the substitution was that extraction at a higher pH would ensure that all VX is present in the non-protonated, extractable form. The second approach was to spike VX directly into the acidified hydrolysate rather than into the buffer. VX is more stable between pH 5 and 9 than at the alkaline buffer pH. Spike addition directly into the hydrolysate also has the added benefit that 100% of the spike is present during extraction as opposed to possibly losing a small amount during transfer of the spiked buffer to the extraction tube. As in the previous section, pH targets were reached by drop-wise addition of concentrated H_2SO_4.

Table 6. Acidified VX Hydrolysate Extraction Recoveries (Buffer[a] VX Spike)

Hydrolysate pH	VX Spike ppb	% Recovery	Average Recovery (%)
5 (4.96)	none	non-detect	-
	160	63.56	69.1 ± 5.0
	160	70.55	
	160	73.22	
7 (6.90)	none	non-detect	-
	135	73.66	72.3 ± 7.1
	160	78.74	
	160	64.56	
9 (8.99)	none	non-detect	-
	160	72.33	71.3 ± 2.9
	160	68.08	
	160	73.58	

[a] 20 g Na_2CO_3/100 mL water (pH 11.4)

Additional extractions of pH 7 hydrolysate were done with the carbonate pH 11.4 buffer to provide a direct comparison to recoveries obtained using the phosphate buffer (~pH 12.5) solutions. Extractions were also performed with both phosphate buffers using pH 5 and pH 9 hydrolysates. The results are listed in Table 7. Extraction recoveries were comparable for the carbonate and phosphate buffers with pH 7 hydrolysate. Similar recoveries using the phosphate buffers were also observed for the pH 5 and pH 9 hydrolysate extractions. Higher extraction recoveries were observed using the acidified hydrolysate VX spike than were observed using the buffer spike (compare Table 7 to Table 6).

No VX was observed in the unspiked pH 9 hydrolysate (flat line for m/z 139). Small m/z 139 peaks were observed in the unspiked pH 7 and pH 5 hydrolysates (estimated ~5 ppb if attributable to VX), but because the qualifying ions 224 and 252 were not present, the data are reported as non-detect.

Table 7. Acidified VX Hydrolysate Extraction Recoveries (Hydrolysate VX Spike)

Hydrolysate pH	Hydrolysate Spike (ppb VX)	Buffer (g/100 mL water)	% Recovery	Average Recovery (%)
5 (5.03)	none	46.8 g K_3PO_4[a]	non-detect	-
	160	46.8 g K_3PO_4	79.4	79.1 ± 0.9
	160	46.8 g K_3PO_4	78.0	
	160	23.4 g K_3PO_4[b]	79.8	
7 (6.98)	none	46.8 g K_3PO_4	non-detect	-
	160	46.8 g K_3PO_4	77.8	79.3 ± 2.5
	160	46.8 g K_3PO_4	77.7	
	160	23.4 g K_3PO_4	77.9	
	160	20.0 g Na_2CO_3[c]	79.4	
	160	20.0 g Na_2CO_3	83.6	
9 (9.05)	none	46.8 g K_3PO_4	non-detect	-
	160	46.8 g K_3PO_4	77.5	80.2 ± 3.4
	160	46.8 g K_3PO_4	79.2	
	160	23.4 g K_3PO_4	84.0	

[a] Buffer pH 12.6 [b] Buffer pH 12.3 [c] Buffer pH 11.4

Two possible sources of VX spike loss are during extraction and SPE cleanup. To gauge the contribution of each, a post-extraction spike was performed on a separate hydrolysate aliquot at pH 7 to investigate what percentage of the ~20% VX loss can be attributed to the SPE cleanup. A VX spike (equivalent to a 160 ng/mL hydrolysate spike) was added to the hexane after extraction and before SPE cleanup. Surprisingly, a lower recovery was observed for the post-extraction spike than for the pre-extraction spike. As shown in Table 8, only 39% VX recovery was observed. The extraction was repeated the following day with a similar result.

Table 8. Post-extraction VX/IPA Spike Using pH 7 Hydrolysate
(46.8 g K_3PO_4/100 mL Buffer, pH 12.6)

Day[a]	Equivalent Hydrolysate Spike (ppb VX)[b]	Equivalent Hydrolysate VX Detected (ppb)	% Recovery
1	160	63.5	39.7
2	160	62.6	39.1

[a] Separate extractions were performed each day.
[b] Spiked after hexane extraction and before SPE cleanup (VX spike in IPA).

The most likely source of this unexpected result is the solvent used for spiking VX. Isopropanol is used for spiking VX into the hydrolysate because it is miscible with the aqueous solution. The same solution was initially used to spike the hexane extract. While only 32 µL of IPA spiking solution was added to 40 mL of washed hexane extract, it is possible this addition of IPA is enough to interfere with the SPE cleanup and cause loss of VX via column breakthrough during hexane elution. To examine the effect of IPA, a 10 µg/mL VX spiking solution was prepared in hexane. Triplicate parallel extractions were then performed with pH 7 hydrolysate using both hydrolysate spiking and post-extraction spiking. Isopropanol solution was used to spike the hydrolysate and hexane solution was used for the hexane extract post-spike. Results are provided in Table 9. These results support the theory that post-spiking with IPA could interfere with the SPE process and also suggest that both the extraction and the SPE cleanup contribute to the ~20% VX loss.

Table 9. Acidified VX Hydrolysate (pH 7) Extraction Recoveries with Pre- and Post-Extraction VX-Spiking (20 g Na_2CO_3/100 mL Buffer, pH 11.4)

Equivalent Hydrolysate Spike (ppb VX)	Spiking Point	% Recovery	Average Recovery (%)
160	Hydrolysate[a]	81.0	81.6 ± 0.9
160	Hydrolysate[a]	81.3	
160	Hydrolysate[a]	82.6	
160	Extract[b]	94.5	89.1 ± 8.3
160	Extract[b]	93.3	
160	Extract[b]	79.6	

[a] Acidified hydrolysate spiked prior to extraction (spiking solution in IPA).
[b] Extract spiked after extraction and before SPE cleanup (spiking solution in hexane).

3.1.3 Evaluation of VX Reformation in Acidified VX Hydrolysate Solutions

Because small peaks were observed for m/z 139 in the chromatograms of pH 7 and pH 5 hydrolysate extracts, further studies were performed to investigate VX reformation in acidified VX hydrolysate. No reportable VX was detected because the qualification ions at m/z 224 and 252 were not present. To investigate whether these ions may grow over time due to possible VX reformation under acidic conditions, a pH 5 hydrolysate solution was prepared by acidification with 93% H_2SO_4 (pH 5.01). This hydrolysate sample was extracted 1 and 7 days after sitting at pH 5 and ambient temperature. K_3PO_4, 25 g/100 mL water, pH ~12.5, was used as the extraction buffer. Ion chromatograms observed for the extract of the 7 day sample are provided in Figure 1. A small broad peak is observed in the VX region (17.19 min) for m/z 139; however, the absence of confirmation ions at m/z 224 and m/z 252 suggests no detectable VX is present in the hydrolysate. The ion at m/z 167 is not shown because of interference. Similar results were obtained for the 1 day sample. These results were also confirmed using an alternative Rtx-5Sil MS column. No detectable VX was observed in the VX hydrolysate after 7 days at pH 5.

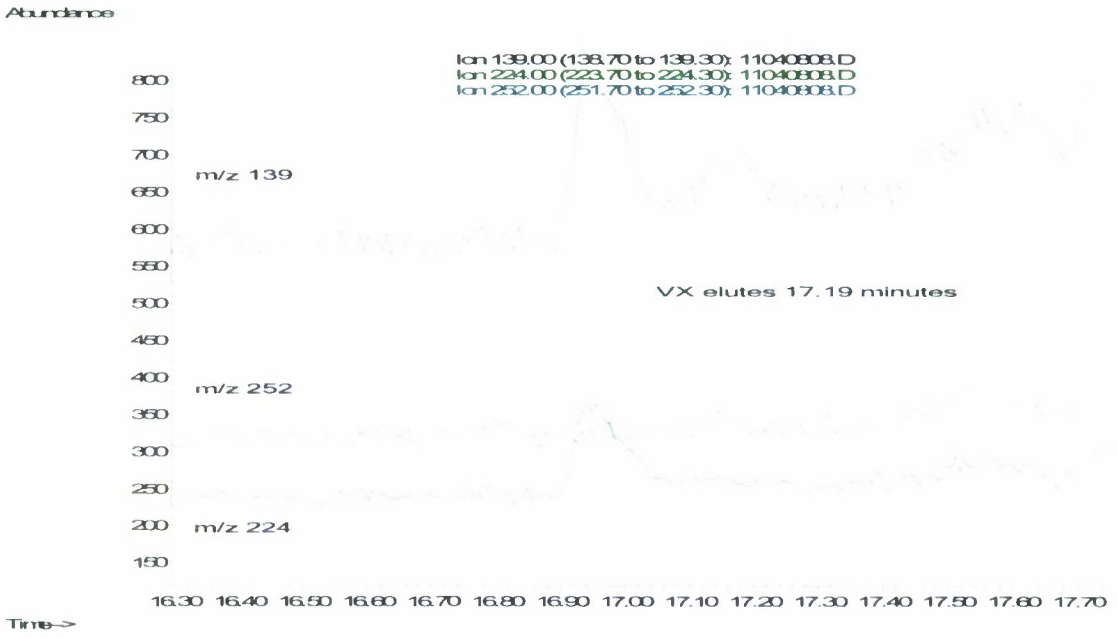

Figure 1. VX Hydrolysate Extract Ion Chromatogram (Extracted after 7 Days at pH 5)

3.2 Application of Modified Method BGCAPP-204 for Analysis of Acidified VX/Energetics Blended Hydrolysate Samples

The studies described here were designed to evaluate the application of the modified method for analysis of trace levels of VX in blended VX hydrolysate/ energetics hydrolysate samples. Since the BGCAPP procedure involves blending VX hydrolysate with energetics hydrolysate prior to feeding the SCWO, it would be desirable to have a working method for analysis of the blend and data indicating that the presence of the energetics does not in some way promote VX formation. All blends were prepared using the plant recipe ratio of 1:2.5 (w:w) for VX hydrolysate:energetics hydrolysate. All blend extractions were performed using 25 g K_3PO_4/100 mL water buffer (pH ~12.5). All analyses were performed using standards prepared in extracts from pH 7 blended hydrolysate.

3.2.1 Initial Blended Hydrolysate Studies at pH 7

Initial testing was performed at pH 7. A blended sample of VX hydrolysate (2 g) and energetics hydrolysate (5 g) was prepared and adjusted to pH 7 with 93% sulfuric acid. The final measured pH was 7.04. One 2 mL aliquot was extracted unspiked to verify the blank was free of VX. Two 2 mL aliquots were each spiked with 160 ppb VX and extracted to ensure VX can be recovered in the presence of energetics hydrolysate. The results are shown in Table 10.

Table 10. VX Hydrolysate/Energetics Hydrolysate Blend (pH 7) Extraction Recoveries

Hydrolysate Blend Spike (ppb VX)	Hydrolysate Blend Observed ng/mL (ppb) VX	% Recovery
0	non-detect	-
160	129.2	80.7
160	142.5	89.1

These preliminary results suggest the method is suitable for the analysis of VX hydrolysate/energetics hydrolysate blend samples. No VX was detected in the blank sample. VX recoveries (80-90%) suggest the energetics hydrolysate does not adversely impact the extraction/SPE cleanup procedure. No analytical interference from the energetics hydrolysate matrix was observed in the VX retention time window.

3.2.2 Blended Hydrolysate Studies at pH 5, 7, 9, and 11

To further test the method and to examine VX reformation as a function of pH, 4 separate VX hydrolysate/energetics hydrolysate blended samples were prepared by adding 2 g VX hydrolysate to 5 g energetics hydrolysate. Three of the four blend samples were adjusted to pH 9, pH 7 and pH 5 using 93% sulfuric acid. The fourth was acidified according to the plant recipe by adding 0.0904 g 35% HCl, 0.0472 g 93% H_2SO_4, and 66.7 mg solid NaCl. Resulting pHs were 9.04, 7.05, 5.03, and 11.26, respectively. Each pH-adjusted sample was split into 3 2 mL aliquots. Duplicate extractions were performed on unspiked samples to investigate VX reformation. One aliquot was spiked at 1Z (160 ppb) prior to extraction to verify VX could be recovered at each pH. The results are listed in Table 11.

Table 11. VX Hydrolysate/Energetics Hydrolysate Blend Extraction Results

Hydrolysate Blend pH	Extraction pH	Hydrolysate Blend Spike (ppb VX)	Hydrolysate Blend Observed ppb VX	% Recovery
5 (5.03)	10.6	0	22.2[b]	-
	-	0	23.8[b]	-
	-	160	137.1	71.3
7 (7.05)	10.7	0	non-detect	-
	-	0	non-detect	-
	-	160	84.8	53.0
9 (9.04)	10.9	0	non-detect	-
	-	0	non-detect	-
	-	160	142.5	89.1
11.26 (Plant Recipe)[a]	11.4	0	non-detect	-
	-	0	non-detect	-
	-	160	122.0	76.3

[a] 2 g VX hydrolysate, 5 g energetics, 0.0904 g 35% HCl, 0.0472 g 93% H_2SO_4, 66.7 mg NaCl.
[b] Based on assumption broad m/z 139 peak in VX region is VX. Additional studies suggest this peak is interference and not VX.

No VX was detected in any of the extracts at pH 7 and above. At pH 5, peaks were observed in the VX retention time window; however, the data presented below indicate the peaks result from matrix interference and not VX. Overall, VX recoveries observed with the VX hydrolysate/energetics blended samples were comparable to those previously obtained for VX hydrolysate alone, although greater variability was observed with the blended samples (53-89% recovery). Previous results obtained for VX hydrolysate using the same method as used here gave recoveries that were consistently in the high 70s and low 80s. The 53% recovery at pH 7 appears to be an anomaly as 81% and 89% recoveries were observed at this pH during the exploratory study (Table 10).

3.2.3 Energetics Hydrolysate Study

To verify that extraction of energetics hydrolysate alone produces an extract free of analytical interferences and that the method can be applied to trace VX analysis of energetics hydrolysate alone, a 2 mL aliquot of the energetics hydrolysate

was extracted according to modified method BGCAPP-204 using K_3PO_4 buffer (25 g/100 mL water, pH ~12.5). The measured pH of the energetics hydrolysate prior to extraction was 7.52 and the extraction pH was 10.83. A second 2 mL aliquot was spiked with 160 ng/mL VX to verify VX can be recovered from this matrix. The results are shown in Table 12.

Table 12. Energetics Hydrolysate Extraction Results

Energetics Hydrolysate Spike (ppb VX)	Energetics Hydrolysate Observed ng/mL (ppb) VX	% Recovery
0	non-detect	-
160	68.0	42.5

No VX was detected in the unspiked energetics hydrolysate. The spiking results verify that VX can be recovered from this matrix using the current method. The low recovery observed likely results from the absence of VX hydrolysate in the matrix. The VX standards used were those prepared for the blended studies and used the blended matrix extract. Although DEAET was added to both sample and standard to enhance the VX signal, the presence of VX hydrolysis products in the standards might be expected to provide some additional enhancement of the standard VX signal relative to the energetics hydrolysate extract VX signal. The observation that the VX peak is broader than that obtained with the blended matrix is an indication that analytical conditions are not optimal in the energetics hydrolysate matrix and further method optimization is warranted. To obtain a more accurate recovery, matrix matching between sample and standard is recommended.

3.2.4 Blended Hydrolysate VX Reformation Studies at pH 5

No reportable VX was detected in any of the acidified blended samples except in the blend at pH 5. At pH 5, a small peak appears in the VX quantitation ion window at m/z 139. The peak is broader than that usually observed for VX, but because the confirmation ions at m/z 224 and 252 also rise above the baseline in the same retention time window, one cannot rule out the possibility that all or a portion of the trace may be attributable to the presence of VX. A chromatogram showing these 3 ions is shown in Figure 2. The ion at m/z 167 is not shown because of interference. The peak eluting in the VX window elutes at 17.07 min compared to 17.09 min for VX within the ±0.15 min acceptable range. At the time of this study, the ion ratio criteria for identification of VX required 2 out of 3 m/z 167/139, 224/139, and 252/139 SIM ratios to be within ±25% of the ion ratios obtained for a mid-range VX standard. An acceptable window of ±50% has also been considered. The ratios observed here for both m/z 224 and m/z 252 fall outside the ±25% widow but within the ±50% window, and therefore would be reported as VX if the wider window were used. To resolve the issue of

whether these peaks were VX or matrix interference, additional studies were performed and are reported below.

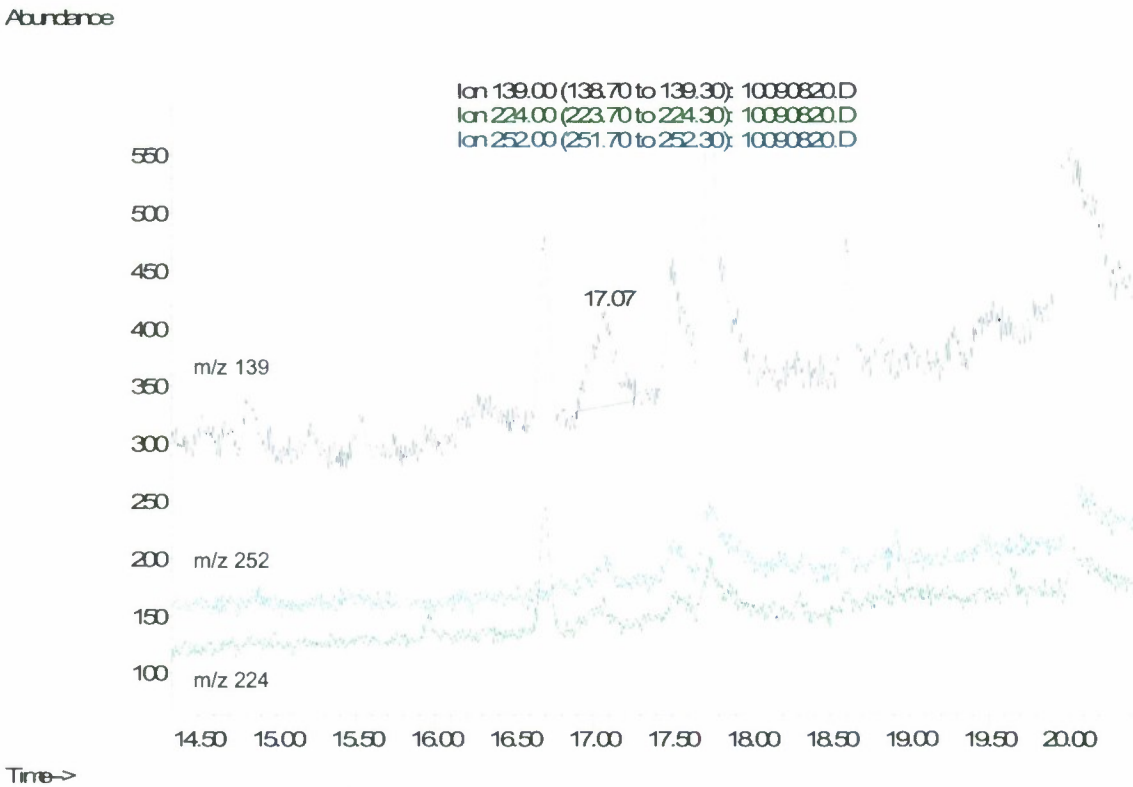

Figure 2. Selected Ion Chromatograms of pH 5 Blend Extract

3.2.4.1 Spiking Studies

To investigate whether the peak in Figure 2 might be interference, the sample was analyzed before and after adding two 20 ppb VX spikes. Analytical results are listed in Table 13 and a visual representation of the m/z 139 ion chromatograms is provided in Figure 3.

Table 13. pH 5 Hydrolysate Blend Extract Spiking Study Results

Extract ID	Extract post-spike (ppb VX)	Extract ng/mL (ppb) VX[b]	Hydrolysate Blend ng/mL (ppb) VX[b]	% Recovery
15605	0	51.3	25.6	-
15605s	20	72.3		104.9
15605sp	40 (15605s + 20)	92.7		102.2

[a] Assuming m/z 139 peak is VX.
[b] Extract volume is half the original hydrolysate blend volume (1 mL extract to 2 mL blend).

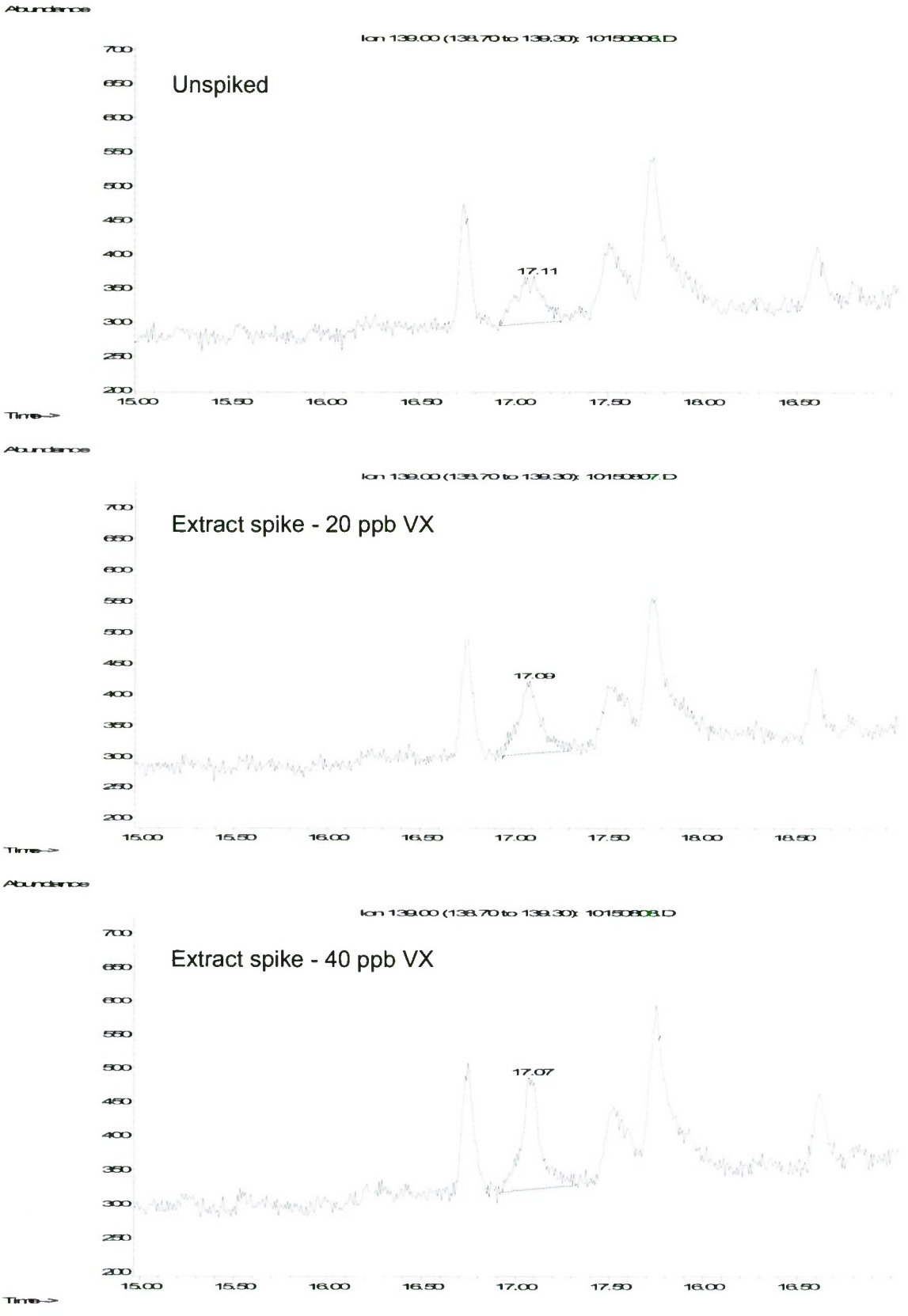

Figure 3. pH 5 Blend Extract Chromatograms (m/z 139) with VX Spikes

The observation that all three VX ions are present in the pH 5 sample and each peak appears to grow as a single peak upon spiking suggests that VX may be present in the sample. However, one cannot rule out that the VX spike elutes precisely on top of a possible interferent and close examination of the spiked chromatographic peaks reveals that the peak could consist of a sharper VX peak on top of a broader interference peak.

3.2.4.2 pH 5 Extract vs. Spiked pH 9 Extract

To provide a comparison of actual VX peak shape to that obtained in the pH 5 extract, the pH 9 blend extract sample was spiked with 40 ppb VX and re-analyzed. The unspiked pH 9 extract chromatogram was relatively peak-free in the VX window. Forty parts per billion were selected to approximate the amount that would be present in the pH 5 extract based on the area of the observed peak. A comparison of the unspiked pH 5 and the unspiked and spiked pH 9 ion chromatograms is shown in Figure 4. The VX peak observed in the pH 9 spiked extract is considerably sharper than the peak observed in the pH 5 extract, suggesting the peak observed in the pH 5 extract may be an interfering peak rather than VX.

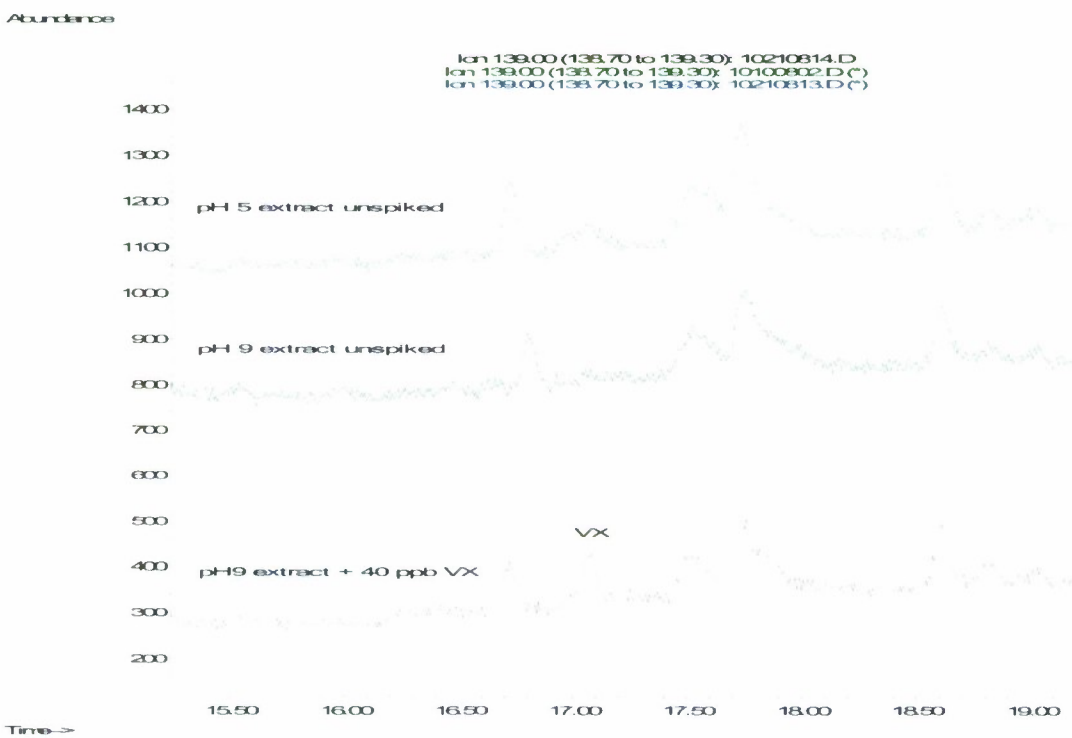

Figure 4. M/z 139 Ion Chromatograms of Unspiked pH 5 Hydrolysate Blend Extract and Unspiked and 40 ppb VX-spiked pH 9 Extract

3.2.4.3 pH 5 Extract - Alternative Column Studies

To provide further evidence that the broad peak observed in the pH 5 extract is not VX, a different phase column was used for analysis of pH 5 blend extract. The Rtx-1701 column was replaced with a Restek 30 m x 0.25 mm Rtx-5Sil MS column with a 1 µm film thickness. During the course of the study VX from VX standards eluted between 14.57 and 14.65 min using this column. Analysis of a 160 ppb VX standard and of the unspiked pH 5 blend extract gave the ion chromatograms shown in Figures 5 and 6, respectively. Analysis of the pH 5 extract reveals a peak at m/z 139 at 14.53 s. As shown, a peak was also observed at m/z 224 but with a slightly shifted retention time. The absence of peaks for the confirmation ions at m/z 167 and m/z 252 provide evidence that VX is not present in this sample and the observed broad peaks are the result of matrix interference.

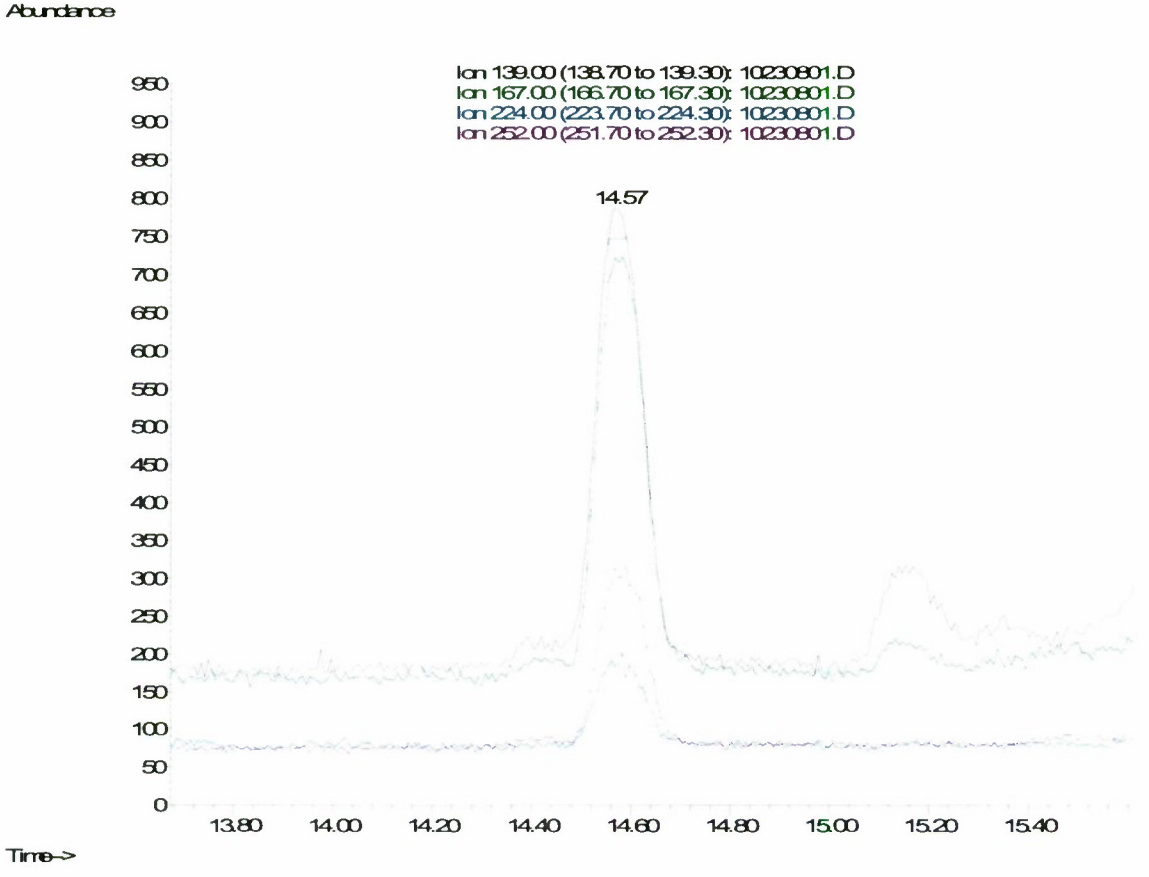

Figure 5. Ion Chromatograms of 160 ppb VX Standard (Rtx-5Sil MS)

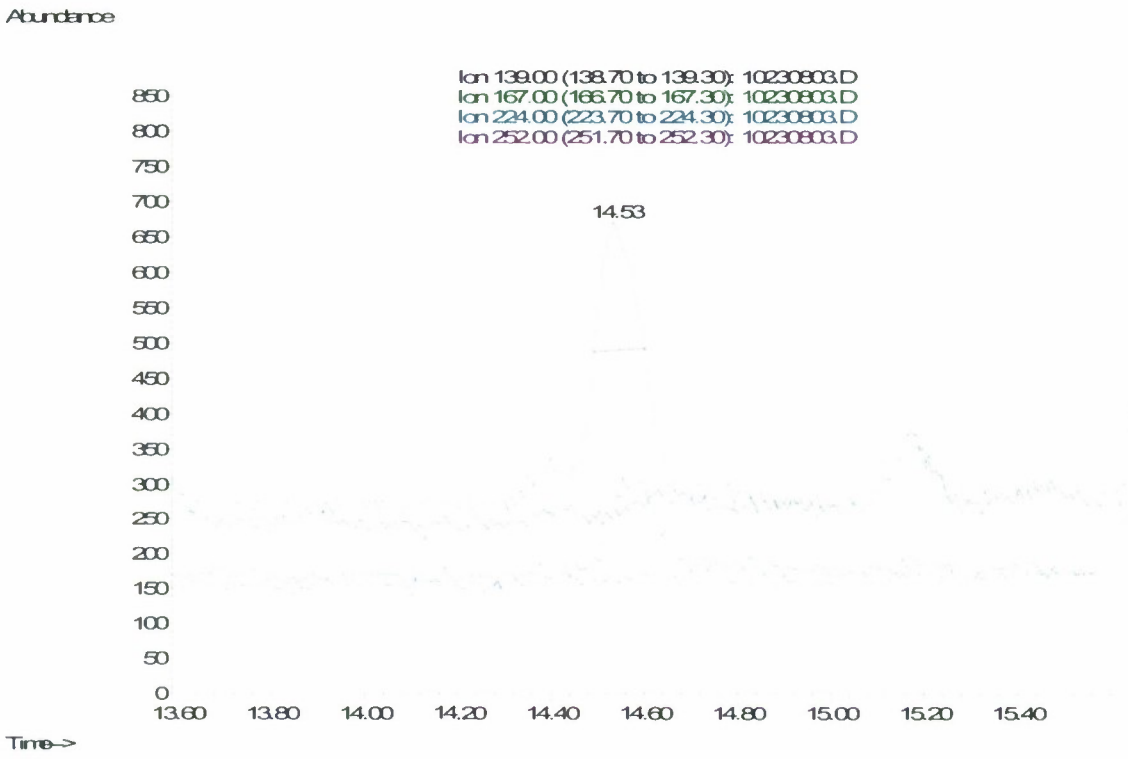

Figure 6. Ion Chromatograms of pH 5 Hydrolysate Blend Extract (Rtx-5Sil MS)

3.3 Comments on Comparability of Lab Procedures to Plant Operations

The following conditions used in the laboratory studies were different than those proposed for use in the plant. None of these differences are expected to impact the fundamental conclusion that VX reformation in VX hydrolysate and blended samples was not observed and is not expected at pH 5 and above.

(1) The composition of the energetics hydrolysate provided for these studies differed from that used in the plant and reportedly contained 528.1 g HCl, 275.6 g H_2SO_4 and 127.8 g NaCl per 1000 mL (1380 g) of energetics hydrolysate. Assuming 35% HCl and 93% H_2SO_4 were used to prepare the mixture, the molar Cl:S ratio of the additives would be 2.78. This is considerably higher than the 1.08 molar ratio required for SCWO operations. Because Cl is in excess, H_2SO_4 was used to adjust the pH for the pH studies to lower this ratio. The resulting ratio, even at pH 5, was still greater than 1:08.

(2) Because of the small sample size, the addition of HCl and H_2SO_4 in this study was completed over a 15 min period. In the plant, 40 min. per additive is allotted for addition of each acid and NaCl.

(3) Additive addition was done at room temperature in the laboratory as opposed to 100 °F in the plant.

(4) Because the laboratory energetics hydrolysate composition was different than the plant composition, the pH of the blend prepared by addition of additives according to the plant procedure may differ from that observed in the plant. The measured pH of the energetics hydrolysate at the time of the study was 7.52. The measured pH of the blend after addition of all additives was 11.26.

(5) In this study, the hydrolysate blend was spiked at the same clearance level as the VX hydrolysate (160 ppb VX). As the VX hydrolysate is diluted 1 part VX hydrolysate to 2.5 parts energetics hydrolysate filtrate, the release level of 160 ppb VX in the plant VX hydrolysate corresponds to a release level of ~46 ppb VX in the blend.

4. CONCLUSIONS

The following conclusions represent significant findings and recommendations related to the modification of method BGCAPP-204 for analysis of trace VX in acidified VX hydrolysate samples:

(1) Three buffers were tested for extraction of VX from acidified VX hydrolysate: 46.8 g K_3PO_4/100 mL water (pH 12.6), 23.4 g K_3PO_4/100 mL water (pH 12.3), and 20 g Na_2CO_3/100 mL water (pH 11.4). All worked equally well and gave comparable VX recoveries (~80%) over the hydrolysate pH range of 5-9. The buffer selected for future studies was 25 g K_3PO_4/100 mL.

(2) VX recoveries obtained by spiking the acidified VX hydrolysate directly were about 10% higher than obtained by spiking the buffer. Spiking directly into the hydrolysate is recommended for analysis of acidified hydrolysate samples.

(3) The applicability of the modified method BGCAPP-204 [using K_3PO_4 buffer (pH ~12.5) instead of KH_2PO_4 buffer (pH 4) and spiking VX directly into acidified hydrolysate rather than buffer] for the detection of low levels of VX in acidified VX hydrolysate/ energetics hydrolysate blended matrices has been demonstrated. The presence of the energetics hydrolysate does not adversely impact the extraction/solid phase extraction (SPE) cleanup procedure. VX recoveries were comparable to those observed with VX hydrolysate alone.

(4) Using isopropanol (IPA) as the VX spiking solvent for hydrolysate and the final extract is preferable, but is unacceptable for spiking the hexane extract prior to SPE cleanup. The IPA appears to interfere with the SPE step.

The following conclusions represent significant findings with respect to VX reformation in acidified VX hydrolysate solutions:

(5) No evidence of VX reformation was observed in VX hydrolysate or VX hydrolysate/energetics hydrolysate (1:2.5) blend over the pH range of 5 to 9. Very small, broad peaks were observed in the m/z 139 window at pH 5 but analysis using an alternative column (Rtx-5Sil MS) confirmed that VX was not present.

(6) No evidence of VX reformation was observed in the blend prepared by addition of additives according to the plant procedure (5 g energetics hydrolysate, 2 g VX hydrolysate, 0.090 g 35% HCl, 0.047 g 93% H_2SO_4, 66.7 mg NaCl, pH 11.26).

(7) No evidence of VX was observed in the energetics hydrolysate alone. VX recovery from this matrix (42.5%) was lower than observed for VX hydrolysate or blended samples containing VX hydrolysate and is likely due to reduced analytical sensitivity relating to the absence of VX hydrolysate matrix in the final extract. The analytical procedures may need to be improved or optimized by using sample/standard matrix matching for analysis of VX in energetics hydrolysate alone.

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